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(54) Title: BIRCH POLLEN ALLERGEN P14 FOR DIAGNOSIS AND THERAPY OF ALLERGIC DISEASES																																																																					
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(57) Abstract																																																																					
<p>The invention provides recombinant DNA molecules which code for polypeptides that exhibit the antigenicity of a P14 allergen of birch <i>Betula verrucosa</i> and other plants of the order Fagales, and for polypeptides comprising at least one epitope thereof, as well as nucleic acids which under stringent conditions hybridize with such DNA sequences or are derivable from such sequences by degeneracy of the genetic code. A method is provided that permits purification of P14 allergens or cross-reactive allergens by means of binding to poly(L-proline). In addition, methods are described for making the proteins and polypeptides coded by these DNA molecules and their use in the diagnosis or therapy of allergic diseases.</p>																																																																					

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BIRCH POLLEN ALLERGEN P14 FOR
DIAGNOSIS AND THERAPY OF ALLERGIC DISEASES

5

1. FIELD OF THE INVENTION

The invention provides recombinant DNA molecules which code for polypeptides, and the polypeptides per se, that have at least one epitope of a P14 pollen allergen of 10 a tree of the order Fagales, particularly birch (Betula verrucosa), or the entire P14 allergen protein, and exhibit the same or similar antigenicity as a P14 allergen. The invention also provides replicable microbial expression vehicles and microorganisms for use 15 in processes for producing such allergenic polypeptides. Methods are provided for purification of P14 allergen as well as for the diagnosis and therapy of allergic diseases using the synthetic polypeptides of the invention.

20

2. BACKGROUND OF THE INVENTION

In the springtime large parts of the populations of Central, Eastern and Northern Europe, America and Australia suffer from allergic symptoms (rhinitis, conjunctivitis, dermatitis and pollen asthma). Proteins 25 which can be isolated from pollen of trees of the order Fagales, in particular from pollen of birch, alder, hazel, hornbeam and oak, are responsible for most of these allergic symptoms (1).

30

At least 10% of the population suffers from pollen allergies at various times and to varying extent. These allergies are mediated by IgE antibodies which react with pollen proteins. The possibility exists for a therapy for pollen allergies by hyposensitization, i.e., 35 by the regular and slowly increasing administration of the prot ins producing the allergy.

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Diagnostic methods for allergic diseases, such as RIA (radioimmunoassay), IRMA (immuno-radiometric assay), RAST (radio-allergosorbent test), ELISA (enzyme-linked immunosorbent assay), magnetic allergoabsorbent test, immunoblots, LIA (luminescence immunoassay), Histamine release assays and others depend greatly upon the availability of pure allergens. Protein extracts from pollen isolated from natural sources are difficult to standardize because preparations vary from batch to batch. For example, they may contain unwanted constituents, and/or certain proteins may be lost in the extraction procedure and be missing from the final separation (2). Clearly, diagnostic tests which employ well defined allergens that can be reproducibly prepared would be superior to tests which employ raw pollen extracts with an insufficiently defined mixture of allergens and other components. Recombinant DNA production of allergenic polypeptides, or allergenic fragments thereof, would allow more reproducible preparations of allergens of defined content for standardized diagnostic and therapeutic methods.

Allergens may be purified to homogeneity from pollen by known protein/chemical methods, for example, by means of affinity chromatography (3). These methods are relatively costly and require pollen as an ill-defined source which cannot be standardized. It would, therefore, be cheaper and more efficient to use recombinant DNA methods to produce an allergenic protein, or fragments of that protein.

Hyposensitization has proved to be an effective therapy in allergic diseases. This therapy consists of parenteral or oral administration of allergens in increasing doses over a fairly long period of time. Like diagnostic methods, it requires pure and well defined allergens. The use of purified recombinant allergens or synthetic peptides would greatly reduce the risk of sensitizing patients to unwanted components.

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3. SUMMARY OF THE INVENTION

This invention concerns pollen allergens, for example, of white birch (Betula verrucosa), called P14. These pollen allergens are immunologically closely related to allergens which occur in pollen of far distantly related plant species, particularly in trees of the Fagales order (birch, alder, hazel, hornbeam and oak), in grasses and weeds. The cross-reactivity of IgE antibodies of patients to these pollen allergens is illustrated in FIGS. 1A and 1B.

The present invention provides recombinant DNA molecules which contain a nucleotide sequence that codes for a polypeptide which exhibits the same or similar antigenic properties as a natural allergen, P14, which occurs in trees of the order Fagales and other pollen producing plants, or a polypeptide which comprises at least one epitope of such allergens. The invention provides the complete cDNA sequence of a P14 allergen and hence the complete deduced amino acid sequence. Additionally, the invention includes (a) nucleotide sequences which hybridize with such a cDNA sequence under high stringency and encode a polypeptide having at least one epitope of a P14 allergen and (b) nucleotide sequences which can be derived from such allergenic polypeptides by degeneracy of the genetic code. This nucleotide sequence can be expressed as a P14 allergen, or as a polypeptide which comprises at least one epitope thereof. In a preferred embodiment, this cDNA sequence contains the whole sequence or parts of the sequence set forth in the Sequence Listing as SEQ ID NO:2.

As concerns their IgE binding, pollens of birch, alder, hazel, hornbeam, oak, grasses and weeds possess similar allergens as the P14 allergen. The present invention therefore relates not only to a P14 allergen of birch, but as well to P14 pollen allergens of other species which are coded by DNA sequences that are able to hybridize with the nucleotide sequence of a birch P14

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allergen under stringent conditions or can be derived from such polypeptide by degeneracy of the genetic code. Hybridization of a polynucleotide with another polynucleotide under stringent conditions requires at least a 60 % identity between such polynucleotides at the nucleic acid level.

Such stringent conditions entail washing of hybridized nitrocellulose filters as follows:

a) For DNA/DNA and DNA/RNA hybridizations: A temperature of 55°C, a salt concentration of 150 mM NaCl and 15 mM Na₃Citrate x 2 H₂O, at pH 7,0, and SDS (Sodium Dodecyl Sulfate) detergent at a concentration of 0,1 % (w/v).

(b) For oligodeoxynucleotides/DNA hybridizations: A temperature of 55°C, a salt concentration of 1M NaCl and 10 mM Na₃Citrate x 2 H₂O, at pH 7,0, and SDS (Sodium Dodecyl Sulfate) detergent at a concentration of 0,5 % (w/v). In this context "oligodeoxynucleotide" refers to an oligomer of a single stranded DNA of up to 100 nucleotides in length.

20 In addition, this invention provides expression plasmids that contain a nucleotide sequence as described above and host cells which harbor these expression plasmids.

This invention also provides compositions containing synthetic polypeptides which exhibit the antigenicity of parts or of the whole of a birch P14 allergen or of allergens of other plants which, because of a high degree (at least 50 %) of amino acid homology, exhibit antigenic cross-reactivity to parts or to all of a birch P14 allergen, i.e., antibodies or cellular antigen binding sites 30 which are actually directed to birch P14 allergen are likewise able to bind to these molecules. These synthetic polypeptides include fusion and nonfusion polypeptides which contain a polypeptide portion that possesses the antigenicity of a part or of all of a P14

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allergen. The method for preparing such synthetic polypeptides comprises the steps of culturing of prokaryotic or eukaryotic host cells which contain an expression plasmid described above and purification of the synthetic polypeptide(s) from the culture.

5 The term "synthetic" here alternatively includes polypeptides which are prepared by cloning and expression of the nucleotide sequences described here or by chemical synthesis of polypeptides encoded by these nucleotide sequences.

10 The synthetic polypeptides which are produced according to this invention exhibit antigenicity the same as or similar to the native allergen. As shown below, a cDNA clone coding for a birch P14 can be used to produce a nonfusion polypeptide which reacts with IgE in the sera of 15 allergic persons. It is therefore possible to use this polypeptide as an antigen in diagnostic tests (such as RAST, ELISA, Immunoblots and others known in the art and referred to above) and as a component of therapeutic agents in hyposensitization therapy.

20 In particular, the synthetic allergens can be used as diagnostic reagents in vitro and in vivo, since their antigenicity corresponds to that of the native P14 pollen allergens and they are therefore able to bind IgE in sera of persons suffering from P14 pollen allergy.

25 It is therefore one of the objects of the present invention to provide a method for the preparation of pollen allergens, in particular for birch P14 allergens, so as to have this family of allergens available for diagnostic tests for detection of the 30 corresponding allergy and, alternatively, for hyposensitization therapy.

35 In addition, as shown below, birch P14 cDNA was expressed in two prokaryotic expression systems in Escherichia coli, and the IgE-binding capacity of the expressed polypeptides, a fusion protein and a nonfusion protein, was demonstrated. This expression can also be

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achieved in any other microorganism (e.g., eukaryotic cells). The IgE-binding capacity was also demonstrated for a partial sequence which was expressed, using lambda gt11, as a β -galactosidase fusion protein. In this way it was demonstrated that this partial sequence represents at least one IgE-binding epitope. In addition, it may be concluded from the results of IgE immunoblots, cross-inhibition tests, clinical tests and Northern (RNA) blots (4-9) (FIGS. 1A, 1B and 3) that homologous IgE-binding polypeptides exist in the pollen of closely related trees of the order Fagales and for distantly related pollen producing plants. For this reason this invention provides polypeptides which exhibit the same or similar antigenicity as the related P14 pollen allergens of birch, alder, hazel, hornbeam, oak, grasses and weeds.

A computer search in the available sequence data banks (EMBL, MIPSX, Swissprot) for proteins whose sequences share homology with birch P14 revealed a significant homology between P14 and a cytoskeletal protein (profilin) which is present in a variety of eukaryotes (10-14) (FIG. 5). This homology raises the possibility of the cross-reactivity of IgE antibodies of patients with human profilin. This autoreactivity has been demonstrated (FIG. 12).

In this way, a molecular system is provided which permits testing the hypothesis: whether autoimmune mechanisms play a role in allergic and atopic diseases. Initial data show that patients whose IgE antibodies react with P14 represent a group that suffers from allergic symptoms during a large part of the year and who do not respond satisfactorily to immunotherapy or conventional therapy. It follows from this that P14, or recombinant or chemically synthesized IgE-binding polypeptides with sequences that match the sequence deduced from P14 cDNA, can be used to characterize a certain group of multivalent allergics as well as a prognostic markers for hyposensitization therapy.

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In addition, this invention presents an efficient method for the production and purification of pollen protein as well as of recombinant or synthetic P14 polypeptide or allergenic fragments thereof. The purification method is based on the affinity of profilin polypeptides for poly(L-proline) (15, 16), and the present inventors' showing of homology between profilins and P14 allergens. Since the binding of pollen protein and of recombinant P14 to poly(L-proline) has been shown herein (FIGS. 8, 9 and 10), a method is thereby provided for immobilizing (and affinity separation of) this allergen. Thus, certain diagnostic tests can be set up (for example, poly(L-proline) may be used instead of an antibody for binding profilin in ELISAs). Likewise, forms of therapy are possible which by means of poly(L-proline) bind P14 and analogous polypeptide allergens. Since there are indications that patients who suffer from autoimmune diseases form antibodies against P14, this polypeptide or homologous polypeptides could be used for diagnosis or therapy of these diseases.

20

4. BRIEF DESCRIPTION OF THE FIGURES

The following figures and description aid in understanding the field and scope of the invention.

25 FIG. 1A: IgE immunoblot: Pollen proteins from birch (B), hornbeam (CA), alder (A) and hazel (C) were separated by means of a 12.5% polyacrylamide electrophoresis and blotted on nitrocellulose. The nitrocellulose was cut into strips (1-5) which were 30 incubated with dilutions of a serum (1:5, 1:10, 1:20, 1:40, 1:80, respectively) of a selected patient whose IgE antibodies recognized most important birch pollen allergens. Arrows and stars indicate molecular weights. Bound serum IgE was detected by means of an autoradiograph 35 of ¹²⁵I-labeled antihuman IgE antibodies of rabbit bound thereto. The IgE-binding proteins of birch, alder, hazel

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and hornbeam matched one another, which demonstrates the similarity of the antigens.

FIG. 1B: IgE immunoblot inhibition: mugwort profilin that had been purified by poly(L-proline) affinity chromatography had been blotted on nitrocellulose after polyacrylamide gel electrophoresis. The 1:10 dilution of the serum from a patient allergic to birch profilin was pre-incubated in lane 1 with control proteins from E. coli, in lane 2 with recombinant birch profilin, in lane 3 with purified profilin from Phleum pratense (grass) and in lane 4 with buffer (negative control) and used for detection of mugwort profilin. Binding of patients' IgE to mugwort profilin can be blocked with recombinant birch profilin and purified grass profilin demonstrating common IgE binding properties of these related proteins. In the control lanes 1 and 4 binding of patients' IgE to mugwort profilin occurs.

FIG. 2: IgE immunoblot: Proteins were separated by means of a 7.5% polyacrylamide gel electrophoresis and blotted on nitrocellulose. Lane B: birch pollen proteins; lane 1: proteins from E. coli Y1089 (lysogenic host); lane 2: proteins of E. coli Y1089 inoculated with the lambda gt11 phage without insert; lane 3: proteins from E. coli Y1089 inoculated with a recombinant phage containing a birch pollen derived cDNA encoding an IgE binding polypeptide (positive control); lane 4: proteins from E. coli Y1089 inoculated with recombinant phages which contain the 3'-portion of P14 cDNA which codes for an IgE-binding epitope (as underlined in FIG. 4); lane 5: proteins from yeast (Saccharomyces cerevisiae). Recombinant β -galactosidase fusion proteins with IgE-binding capacity whose molecular weights were between 115 and 130 kD (lanes 3 and 4) were detected with ^{125}I -labeled antihuman IgE antiserum from rabbit. No comparable IgE binding takes place in lanes 1, 2 and 5, while lane B shows the patient's IgE-binding profile with birch pollen proteins.

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FIG. 3: Northern (RNA) blot: Ten μ g pollen RNA of alder (lane A), birch (lane B) and hazel (lane C) and as a marker RNA of E. coli (lane M) were blotted on nitrocellulose. The part of the P14 cDNA underlined in FIG. 4 hybridizes with pollen mRNA of alder, birch and 5 hazel and under stringent conditions ($0.75 \times SSC$, 0.1% SDS, $50^{\circ}C$) produces a signal at 800 bases (indicated by an arrow). The position of ribosomal bands is indicated by "28S" and "18S".

FIG. 4: cDNA sequence of birch P14. The coding 10 region begins with ATG (nucleotides 80-82) and ends with the stop codon TAG (nucleotides 479-481). The deduced amino acid sequence is illustrated under the DNA sequence. The P14 sequence, which within a fusion protein is able to bind IgE of patients and therefore represents at least one 15 epitope, is shown underscored (see Section 5.4).

FIG. 5: Comparison of the derived amino acid sequence of birch P14 with the amino acid sequences of profilins of human (13), calf (14), mouse (12), yeast (11) and Acanthamoeba (10). Identical amino acid residues are 20 marked. The percentage of identical amino acid residues between P14 protein of birch and homologs amounts to 30% for human protein, 28% for homologous proteins of calf and mouse, 26% for yeast protein and 25% for Acanthamoeba protein.

FIG. 6: Western (protein) blot of a 25 polyacrylamide gel probed with IgE antibodies of patients with tree pollen allergy. Lane 1: proteins of E. coli JM105 without any plasmid; lane 2: proteins of E. coli JM105 with the plasmid pKK223-3 without an insert; lanes 3 and 4: proteins of E. coli JM105 with that plasmid 30 derived from pKK223-3 which expresses the P14 protein of the inserted cDNA as nonfusion protein; lane 5: E. coli AR58 proteins; lane 6: E. coli AR58 with the plasmid pEXB without an insert; lanes 7 and 8: extracts from E. coli 35 AR58 transformed with the plasmid derived from pEXB which

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expresses P14 cDNA as fusion protein; lane 9: birch pollen protein extract (positive control).

FIG. 7: Sera from various allergic patients were tested for their IgE reactivity with respect to recombinant P14 which was expressed in pKK223-3. Patients (lanes) D, E, F, I, J, and P show IgE binding to the recombinant P14. Lane R is a serum pool from non-allergic individuals. The recombinant P14 was not purified and, therefore, reactivity of patients' IgE with proteins from E. coli was seen.

FIG. 8: Coomassie stained polyacrylamide gel. Lane M: molecular weight marker; lane 1: total pollen proteins of birch; lane 2: birch pollen proteins from which P14 was removed by the affinity method (flow through); lanes 3, 4 and 5: eluted P14. Proteins were applied to the gel and stained to indicate migration. As can be seen from lanes 3, 4 and 5, P14 can be purified by affinity chromatography to poly(L-proline) sepharose.

FIG. 9: IgE immunoblot: A probe of proteins obtained in the same way as in FIG. 8 was transferred to nitrocellulose and incubated with serum IgE of a patient who recognizes most birch pollen allergens. Lanes 1 - 5 contain the same material as in FIG. 8; lane 6 contains the molecular weight marker. This immunoblot shows that birch profilin can be purified to apparent homogeneity from other allergens.

FIG. 10: Coomassie-stained polyacrylamide gel. Lane M: molecular weight marker; lane 1: total proteins of E. coli JM105 with the plasmid derived from pKK223-3 that expressed the P14 cDNA; lane 2: protein fraction after removal of the recombinant P14 by affinity chromatography to poly(L-proline) sepharose; lanes 3 and 4: purified recombinant P14-eluted fractions.

FIG. 11: Coomassie-stained polyacrylamide gel. Lane M: molecular weight marker; lane 1: total protein from E. coli JM105 with the plasmid pKK223-3 without insert; lane 2: protein fraction after poly(L-proline)

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purification; lanes 3, 4 and 5: eluted fractions. These results show that no protein with similar properties to P14 can be isolated from the expression system without insert.

FIG. 12: IgE immunoblot: Purified human profolin was loaded on a 12% polyacrylamide gel and blotted on nitrocellulose. Strips of the nitrocellulose were cut and incubated as follows: Strip 1 was incubated with serum IgE of a patient who recognized besides P14 and the major birch pollen allergen, Bet v I, allergens in the molecular range between 30 and 90 kD. Strip 2 was incubated with serum IgE from a patient who recognized only P14 in birch pollen extracts; strip 3 was incubated with serum from a patient whose serum IgE was directed only against Bet v I; strip 4 was incubated with the serum from a patient allergic to mites; strip 5 with serum from a group of nonallergic donors and strip 6 shows the buffer control. IgE binding was detected with a ¹²⁵I-labeled antihuman IgE antiserum of rabbit. Cross-reactivity was shown for strips 1 and 2. This data demonstrates that serum IgE that reacts with birch pollen also cross-reacts with human profolin.

FIG. 13: IgE-inhibition:

Purified celery profolin was subjected to SDS-Page, blotted to nitrocellulose (1 μ g/cm). Nitrocellulose strips where incubated with 1:10 dilutions of sera from birch pollen profolin allergic patients (lanes 1-3), from patients allergic to the major birch pollen allergen Bet v I but not to profolin, a serum pool of nonallergic individuals (lane 4) and with buffer without addition of serum (lane 5). The

serum dilutions where preincubated with 5 μ g of purified recombinant birch profilin each (rP), 5 μ g of BSA (BSA), and with serum dilution buffer(P). Binding of IgE of the patients 1-3 to celery profilin can be blocked with purified recombinant birch 5 profilin indicating common IgE epitopes of birch and celery profilin.

FIG. 14: Immunoblots:

10 Purified celery profilin (C) and recombinant birch profilin (rP) is recognized by the rabbit anti celery profilin antibody (R: lane 1). Recombinant birch profilin also binds patients IgE (IgE: lane 1). No binding is seen in lane 2 (buffer control without addition of antibody or serum).

15

FIG. 15: Immunoblots:

Purified profilin from rye (*Secale cereale* S) and from mugwort 20 (*Artemisia vulgaris* M) binds the rabbit anti celery profilin antibody (lanes 1) whereas in the buffer control (lane 2) no binding was found.

Bound rabbit antibody in Figures 14 and 15 was detected with 125 25 J donkey anti rabbit antibody from Amerham, UK. Bound serum IgE in Figures 13 and 14 was detected with 125 J rabbit anti human IgE from Pharmacia, Sweden.

FIG. 16: cDNA and deduced amino acid sequence of profilin from pollen of timothy grass (*Phleum pratense*)

5

FIG. 17: IgE-binding of patients IgE to plaquelifts of lambda gt 11 phages containing the cDNA insert encoding timothy grass profilin

30 grass pollen allergic patients were tested for IgE binding to
10 lambda gt 11 phages that express profilin from timothy grass which was bound to nitrocellulose sectors. Sector 31 shows a serum pool from non allergic individuals and sector 32 the buffer control without addition of serum. Serum IgE of patients 2, 6, 7, 8, 9, 10, 11, 12, 15, 17, 18, 21, 23, 26, 27, 28, 29 and 30 bound to the
15 recombinant timothygrass profilin expressed in lambda gt 11. All these patients also displayed IgE reactivity to birch profilin. Patients 1, 3, 4, 5, 13, 14, 16, 19, 20, 22, 24, and 25 who were allergic to other grass pollen allergens did not show any IgE reactivity to timothy grass profilin. Bound serum IgE was detected as described
20 in Fig. 13.

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5. EXAMPLES

The invention can be understood by reference to the following examples:

5 5.1. Construction of the cDNA gene bank

Pollen (Allergon AB Engelholm, Sweden) which was examined for purity by means of light and electron microscopy, was used for the isolation of polyadenylated RNA (17, 18). cDNA synthesis was carried out with oligo-
10 dT and random primers (19, 20), the ends of the cDNA were cleanly digested with T4-polymerase and provided with EcoRI-linkers. The cDNA with linkers was ligated and packed in dephosphorylated lambda gt11 arms (21). A cDNA gene bank of 800,000 independent clones was produced.

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5.2. Screening of the cDNA gene bank

IgE screening of the birch pollen cDNA gene bank was performed as described (22). IgE-binding clones were enriched and phage DNA was prepared therefrom (23). The inserts were cut out with EcoRI and the fragments were 5 subcloned in the plasmid pUC18 (24). The DNA sequence of a clone was obtained (25). Although it was complete at the 3'-terminus (poly-A tail), it lacked a part of the 5'-terminus including the start codon as well. This partial sequence is underscored in FIG. 4. Therefore the original 10 gene bank was again screened with oligodeoxynucleotides which were complementary to the coding region (26) and two independent clones were obtained.

5.3. RNA (Northern) blots

15 Ten µg of total RNA from pollen of alder, birch and hazel were separated by means of a denaturing gel electrophoresis and blotted on nitrocellulose (27, 28). A P14 cDNA probe, as underlined in FIG. 4, was ³²P-labeled by means of random priming (29). Prehybridization and 20 hybridization were carried out by standard methods (23). The blots were washed with 0.75xSSC (20xSSC = 3M NaCl, 0.3M Na citrate, pH 7.0), 0.1 % SDS (sodium dodecyl sulfate) at 50°C and autoradiographed (Hyperfilm MP, Amersham, London, UK).

25

5.4. Expression of Birch P14 cDNA

5.4.1 Expression of the 3'-terminus of the cDNA in lambda gt11 phages (FIG. 2)

30 An incomplete cDNA clone which codes for a part of P14 was obtained by means of IgE screening (22) as described in Section 5.2. The lysogenic E. coli strain Y1089 was inoculated with recombinant lambda gt11 phages, containing an insert as underlined in FIG. 4, and the 35 6-galactosidase fusion protein was recovered from the mixture (19). The construction would predict that a fusion protein having a molecular weight of 116 kD would

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be produced. The mixture was subjected to electrophoresis on a 7.5% polyacrylamide gel and was blotted on nitrocellulose. The fusion protein was detected by means of IgE antibodies in patient serum and an iodine-labeled rabbit antihuman IgE antibody (Pharmacia, Uppsala, Sweden) 5 (FIG. 2). As shown in FIG. 2, a fusion protein having a molecular weight of between 115 kD and capable of binding to IgE antibodies was observed.

10 5.4.2. Expression of complete P14 cDNA
 as fusion and nonfusion protein

The complete cDNA that codes for P14 contains a prokaryotic ribosome binding site (Shine-Dalgarno sequence (30)) and was inserted into the EcoRI sites of plasmids pKK223-3 (31) or pEXB (32) to obtain P14 as a nonfusion 15 protein or a fusion protein of P14 with the lambda cII protein. IgE-binding clones were obtained by means of serum IgE and a colony screening method (33) and were examined by means of DNA restriction analysis. Recombinant proteins were tested for their binding 20 capacity with respect to patient IgE antibodies as described (22) (FIG. 6).

25 5.5. Purification of birch pollen P14
 and recombinant P14

P14 from birch pollen and recombinant P14 were purified by means of an affinity method by a batch process (cf. 15, 16) which is suitable for the profilins of Acanthamoeba (10), yeast (11) and man (13). Birch pollen and E. coli cells, which contain the plasmid that codes 30 for P14, were lysed in PHEM-TX buffer (2x PHEM-TX: 120 mM PIPES (piperazine-1,4-bis(2-ethanesulfonic acid)), 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 20 mM EGTA (ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid), 4 mM MgCl₂, 10 mM glucose, 20 µg/ml leupeptin, 156 µg/ml benzamidin, 80 µg/ml aprotinin, 1 mM PMSF (phenylmethyl sulfonyl 35

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- fluoride), 1.5% Triton-X100, pH 7.2) and the lysate was centrifuged for one hour at 65000 x g at 4°C. The supernatant was incubated overnight at 4°C with poly(L-proline) coupled to BrCN-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). Then the affinity matrix 5 was washed three times with a double volume each time of TBS-ATP (20 mM TRIS, 150 mM NaCl, 0.5 mM ATP (adenosine triphosphate), pH 7.6) and then eluted for five minutes at room temperature with a double volume of elution buffer I (TBS-ATP with 2M urea). The supernatant was collected. 10 The procedure was repeated twice with elution buffer II (TBS-ATP with 6M urea) and the supernatants were dialyzed against distilled water at 4°C. The dialysates, which contained the proteins, were lyophilized and analyzed by means of polyacrylamide gel electrophoresis and IgE 15 immunoblot (FIGS. 8, 9, 10 and 11).

5.6. IgE-binding capacity of a protein expressed from a fragment of P14 cDNA which contains an IgE-binding epitope

20 The 3'-region of P14 cDNA (bp 419-478) was cloned in the EcoRI site of lambda gt11 and expressed as an IgE-binding polypeptide (21) as shown in FIG. 2. The β -galactosidase fusion protein (lane 4) bound strongly to IgE of the patient, while the control lanes 1 and 2 25 exhibited no IgE binding for the proteins of E. coli Y1089 and the proteins of E. coli Y1089 which were inoculated with lambda gt11 phages without an insert. This example shows that a partial cDNA clone which codes for a protein having at least one epitope of the P14 molecule was 30 obtained. It follows from this that partial cDNA clones which code for such incomplete P14 polypeptides may be useful for a therapy or diagnosis in a way similar to the complete P14 molecule or homologous proteins.

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5.7. Demonstration of polynucleotides
and polypeptides homologous to P14
within the order Fagales

The Northern (RNA) blot (FIG. 3) shows that the P14 cDNA sequence (polynucleotide underlined in FIG. 4) is able to cross-hybridize with pollen mRNA from alder and hazel under stringent conditions (requirements of stringency are defined in 3. Summary of the Invention). Therefore, the sequence homology of the corresponding allergens of trees of the order Fagales can already be demonstrated at the nucleic acid level. FIG. 1A already showed a similar IgE-binding capacity of proteins of alder, hazel and hornbeam homologous to P14. It follows from this that P14 cDNA codes for polypeptides of similar IgE-binding capacity and antigenicity to closely related tree pollen allergens.

5.8. Sequence analysis

FIG. 4 shows the sequence of the cDNA that codes for birch P14, and the deduced amino acid sequence of the coding region. It contains the complete protein coding region. The sequence of the peptide that, coupled to 6-galactosidase, represents an IgE-binding epitope is underlined in the figure (see Example 5.4).

FIG. 5 illustrates the sequence homology between the P14 protein of birch and of human, mouse, calf, yeast and Acanthamoeba profilins (13, 12, 14, 11, 10).

The cross-reactivity of patient IgE with birch P14 and human profilin is shown in FIG. 12. Similar chemical properties of these related proteins were likewise shown by their common affinity to poly(L-proline) (FIGS. 8 and 10). These data indicate that the profilins of species which are as far apart in evolutionary terms as humans and birch are able to act as cross-reactive panallergens that may lead to an IgE autoimmune reactivity in patients.

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5.9. Expression of P14 coding cDNA in
E. coli as fusion or nonfusion
protein and detection of IgE-binding
capacity of these polypeptides

The polynucleotide given in FIG. 4 (nucleotides 1-710) that codes for birch P14 was inserted in the 5 plasmid pKK223-3 so that a recombinant nonfusion protein (31) could be prepared, while a recombinant fusion protein was produced in the plasmid pEXB (32). The reactivity of these peptides with patient IgE is shown in FIG. 6. Control protein extracts of E. coli in lanes 1, 2, 5 and 6 10 do not bind IgE, while recombinant birch P14 expressed as a nonfusion protein (lanes 3 and 4) and as a fusion protein (lanes 7 and 8) does bind IgE.

In FIG. 7, sera from persons allergic to birch pollen (A-K), to grass pollen (L-N) and to mugwort (O-Q) 15 and of a pool of nonallergic individuals (R), all of whom had been selected according to their case history, RAST and skin test, were tested for their IgE-binding capacity with recombinant birch P14. IgEs of Sera D, E, F, I, J and P bound to P14 expressed in pKK223-3.

It follows from this that this invention provides a polynucleotide that codes for polypeptides which have similar antigenicity and similar IgE-binding capacity to the P14 protein of birch when the 20 polynucleotide is inserted in the correct reading frame of a variety of expression systems. The IgE-binding properties of these polypeptides were demonstrated for sera from patients who exhibit allergic reactions to various pollens and hence point to the great clinical significance of these polypeptides (FIG. 7).

30

5.10. Purification of P14 from pollen
and of recombinant P14 from E. coli

As described above, this invention provides a 35 simple method for purifying natural as well as recombinant P14. The Coomassie-stained polyacrylamide gel in FIG. 8 shows that pure P14 (lanes 3, 4 and 5) can be separated

-20-

from total pollen protein (lane 1). The proteins which do not bind to poly(L-proline)-Sepharose are also shown (lane 2). The effectiveness of this purification method was monitored by means of IgE immunoblotting (FIG. 9), and for this purpose serum from a patient who recognizes most 5 birch pollen allergens with IgE antibodies (lane 1) was used. After application of the affinity method, almost no P14 can be found (lane 2), while purified P14 was obtained in lanes 3, 4 and 5.

FIG. 10 shows a polyacrylamide gel which 10 demonstrates the purification of recombinant P14 from E. coli JM105 that is transformed with the plasmid pKK223-3, which carries the P14 coding sequence. Recombinant P14 (lanes 3 and 4) was purified from the total proteins by 15 affinity chromatography to poly(L-proline) sepharose (lane 1), the remaining proteins being shown by lane 2. FIG. 11 shows that no homologous protein from E. coli JM105 transformed with pKK223-3 without insert is obtained by means of the method used.

As FIGS. 9, 6 and 7 show, the purified protein 20 (from birch and E. coli) retains its IgE-binding capacity. This example thus shows that the present invention likewise provides a simple and rapid purification method for P14 both as natural and recombinant polypeptide. Using poly(L-proline) to purify, both natural and 25 recombinant P14 retain their antigenicity and IgE binding capacity. In addition, the method offers the opportunity to immobilize (and separate by affinity means) the immunologically active polypeptide.

30 5.11. IgE reactivity of allergic and
 atopic patients with human profilin

Various patient sera were selected as follows (FIG. 12): Patient 1 shows IgE antibodies which are directed against most birch pollen allergens, including 35 Betyl and P14, patient 2 shows IgE binding only with P14 and patient 3 only with Betyl. Patient 4 is a person

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allergic to house dust mite and the serum pool 5 was made up of nonallergic individuals. Strip 6 is the buffer control. All these sera were tested for their IgE-binding capacity with nonrecombinant and recombinant P14 and human profilin. Those patients (FIG. 12), who recognized the
5 nonrecombinant P14 from birch as well as the recombinant P14 from E. coli, also had IgE antibodies against human profilin. For this reason, this invention for the first time gives indications on the molecular level that autoimmune mechanisms might play a role in atopic and
10 allergic diseases. Since patients with other autoimmune diseases form antibodies against P14, this invention should provide a diagnostic marker for these diseases.

15 5.12. Correlation of case histories of
 atopic and allergic patients with
the binding of IgE antibodies to P14

The case histories of patients who form IgE antibodies against P14 show that all of them suffer from severe allergic symptoms which are caused by a great
20 variety of allergens (tree and grass pollen, mite, cat and dog allergens), that they have an elevated total IgE level and show an unsatisfactory course in hyposensitization therapy. It follows from this that a positive reaction of
25 the serum IgE of patients with P14 is usable as a good marker for the differentiation of certain groups of atopic and allergic patients.

5. 13. Demonstration of common IgE-epitopes between profilins
in food (celery) and pollen P14 allergen (birch)

The IgE-inhibition experiment shown in Figure 13 shows that

- 5 there is common IgE-binding capacity of proteins homologous
to the P14 allergen in pollens and food. Purified recombinant
birch profilin, when added to the patients' serum in the fluid
phase before the serum is incubated with the nitrocellulose
bound celery profilin, is able to completely block the binding of
10 patients' IgE to celery profilin. This indicates that the P14
allergen of birch (birch profilin) contains all IgE epitopes that
can be found in celery profilin. Recombinant birch P14 allergen
is therefore suitable not only for diagnosis and therapy of
pollen allergies but also for food allergies.
- 15 Common antigenicity of birch pollen P14 allergen and food
profilins is also shown by crossreactivity of a rabbit anti celery
profilin antibody with the pollen P14 allergen (Figs. 14 and 15).

5. 14. Sequence similarity and common IgE-binding capacity of
20 pollen P14 allergen from white birch (*Betula verrucosa*) and
the homologous P14(T) allergen from timothy grass (*Phleum
pratense*).

- Amino acid sequence identity of birch P14 allergen and
25 timothy grass P14(T) allergen is 77%. The cDNA encoding the
P14(T) allergen from timothy grass was obtained by screening
a cDNA library, which was constructed from pollen of timothy

grass in the same way as described for the cDNA library from birch pollen, with patients' IgE as described for the P14 allergen of birch. The cDNA sequence of the clone encoding the P14(T) allergen of timothy grass is shown in Fig. 16. and as sequences 9-11 in the sequence listing. All patients' sera that bound with their IgE to birch profilin also bound with their IgE to sectors of nitrocellulose filters containing plaquelifts of immunopositive lambda gt11 phages into which the cDNA encoding timothy grass P14(T) allergen had been inserted (Fig. 17). This shows that the proteins related to the P14 allergens by high homology also are immunologically cross reactive with patients' IgE antibodies.

6. METHODS OF ADMINISTRATION

The present invention covers the use of P14 synthetic polypeptide allergens to hyposensitize or desensitize a mammal. Such polypeptides can be administered to a human subject either alone or in combination with pharmaceutically acceptable carriers or diluents, in accordance with standard pharmaceutical practice.

The method of hyposensitization involves or could involve the successive parenteral, oral, nasal,

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inhalant or rectal administration of incremental doses of the P14 allergen. The term parenteral as used herein includes subcutaneous, intravenous or intramuscular injections.

A range from 1 picogram to 10 milligrams per 5 application can be used. The diluents and carriers can be chosen by those skilled in the art according to commonly accepted galenic procedures.

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8. SEQUENCE LISTING

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10 for Diagnosis and Therapy of Allergic Diseases

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(IBM-PC Compatible)
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WordPerfect 5.1

25 (vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: 8
(B) FILING DATE:
(C) CLASSIFICATION:

- 30 (vii) PRIOR APPLICATION DATE:
(A) APPLICATION NUMBER: 07/353,844
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-30-

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 700 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA or mRNA

10 (iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE:

15 (A) ORGANISM: Betula verrucosa

15 (vii) IMMEDIATE SOURCE:

(A) POLLEN FROM ALLERGON AB, ENGELHOLM,
SWEDEN

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE: Not Applicable

20 (x) PUBLICATION INFORMATION: Not Applicable

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAGAGAAAGC GAAAGCTCTC CGCCACARCA AAACGAAGTA GAAGAAGRAG AGTGAGCAAG 60

AGACAGAGGG AAGAGGAAAA TGTCGTGGCA AACGTACGTG GATGAACATT TGATGTGCGA 120

TATCGACGGG CAAGCCAGCA ACTCGCTGGC ATCTCGGATC GTCCGGTCACG ATGGCTCTGT 180

GTGGGCCAG AGCTCTTCCT TCCCCACAGTT TAAGCCTCAAG GAAATCACTG GTATCATGAA 240

25 GGACTTTGAG GAGCCGGGTC ATCTTGCCTCC GACGGGCTTA CACCTTGGGG GCATAAAAATA 300

CATGGTCATC CAGGGAGAGG CTGGTGTGT CATCCGTGGA AAGAAGGGAT CTGGAGGTAT 360

TACTATAAAG AAGACTGGTC AAGCTCTCGT TTTTGGCATC TATGAAGAGC CTGTGACACC 420

AGGACAGTGC AACATGGTTG TTGAGAGGTT GGGGGATTAC CTTATTGACC AGGGCCTGTA 480

GGCAAAAGTC TATCATCATT TGGGGCTTAA TTGTTTTTTT TTTTTTTTG CTCTTATTCC 540

30 CTTTGATTTC GGTCCAAGT GTGCATCGAT CTTCATTTGA AAGCCTTAAA TTGGCAGTGA 600

AGTTGTTGCA GACAATAACC ATGTGAGAAC TAAAACATT GTCTTGTGTT TGGTTGTTG 660

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 700

(3) INFORMATION FOR SEQ ID NO:2:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 nucleotides

-31-

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: liner
5 (ii) MOLECULE TYPE: cDNA or mRNA
(iii) HYPOTHETICAL: No
(iv) ANTI-SENSE: No
(v) FRAGMENT TYPE: Not Applicable
(vi) ORIGINAL SOURCE:
10 (A) ORGANISM: Betula verrucosa
(vii) IMMEDIATE SOURCE:
(A) POLLEN FROM ALLERGON AB, ENGELHOLM,
SWEDEN
(viii) POSITION IN GENOME: Not Applicable
(ix) FEATURE: Not Applicable
(x) PUBLICATION INFORMATION: Not Applicable

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGTCGTGGC AACCGTACGT GGATGAACAT TTGATGTGCG ATATCGACGG GCAAGCCAGC 60
AACTCGCTGG CATCTGCGAT CGTCGGTCAC GATGGCTCTG TGTGGGCCCA GAGCTCTTCC 120
TTCCCCACAG TTAAGCCTCA GGAAATCACT GGTATCATGA AGGACTTTGA GGAGCCGGGT 180
CATCTTGCTC CGACGGGCTT ACACCTTGGG GGCATAAAAT ACATGGTCAT CCAGGGAGAG 240
GCTGGTGCTG TCATCCGTGG AAAGAAGGGA TCTGGAGGTA TTACTATAAA GAAGACTGGT 300
20 CAAGCTCTCG TTTTGGCAT CTATGAAGAG CCTGTGACAC CAGGACAGTG CAACATGGTT 360
GTTGAGAGGT TGGGGGATTA CCTTATTGAC CAGGGCTG 399

(4) INFORMATION FOR SEQ ID NO:3:
25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Applicable
(D) TOPOLOGY: linear
30 (ii) MOLECULE TYPE: peptide
(iii) HYPOTHETICAL: No
(iv) ANTI-SENSE: Not Applicable
(v) FRAGMENT TYPE: Not Applicable
(vi) ORIGINAL SOURCE:
35 (A) ORGANISM: Betula verrucosa
(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE:

(D) OTHER INFORMATION: Amino acid sequence identity with profilin of other organisms is as follows:
30% with human profilin, 28% with calf and mouse, 26% with
5 yeast and 25% with Acanthamoeba

(x) PUBLICATION INFORMATION: Not Applicable

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Cys Asp Ile Asp				
1	5	10	15	
Gly Gln Ala Ser Asn Ser Leu Ala Ser Ala Ile Val Gly His Asp Gly				
10	20	25	30	
Ser Val Trp Ala Gln Ser Ser Phe Pro Gln Phe Lys Pro Gln Glu				
35	40	45		
Ile Thr Gly Ile Met Lys Asp Phe Glu Glu Pro Gly His Leu Ala Pro				
50	55	60		
Thr Gly Leu His Leu Gly Ile Lys Tyr Met Val Ile Gln Gly Glu				
15	65	70	75	80
Ala Gly Ala Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Ile				
85	90	95		
Lys Lys Thr Gly Gln Ala Leu Val Phe Gly Ile Tyr Glu Glu Pro Val				
100	105	110		
Thr Pro Gly Gln Cys Asn Met Val Val Glu Arg Leu Gly Asp Tyr Leu				
115	120	125		
Ile Asp Gln Gly Leu				
130				

25 (5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 140 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Not applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE:

35 (A) ORGANISM: MOUSE (Murine)

(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

-33-

(ix) FEATURE:

(D) OTHER INFORMATION: 28% identical with
the P14 allergen of birch (Betula verrucosa)

(x) PUBLICATION INFORMATION: ref. 12

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

5 Met Ala Gly Trp Asn Ala Tyr Ile Asp Ser Leu Met Ala Asp Gly Thr
1 5 10 15
Cys Gln Asp Ala Ala Ile Val Gly Tyr Lys Asp Ser Pro Ser Val Trp
20 25 30
Ala Ala Val Pro Gly Lys Thr Phe Val Ser Ile Thr Pro Ala Glu Val
35 40 45
Gly Val Leu Val Gly Lys Asp Arg Ser Ser Phe Phe Val Asn Gly Leu
50 55 60
10 Thr Leu Gly Gly Gln Lys Cys Ser Val Ile Arg Asp Ser Leu Leu Gln
65 70 75 80
Asp Gly Glu Phe Thr Met Asp Leu Arg Thr Lys Ser Thr Gly Gly Ala
85 90 95
Pro Thr Phe Asn Val Thr Val Thr Met Thr Ala Lys Thr Leu Val Leu
100 105 110
Leu Met Gly Lys Glu Gly Val His Gly Gly Leu Ile Asn Lys Lys Cys
115 120 125
Tyr Glu Met Ala Ser His Leu Arg Arg Ser Gln Tyr
130 135 140
15

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

20 (B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: No

25 (iv) ANTI-SENSE: Not applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Calf (Bovine)

(vii) IMMEDIATE SOURCE: Not applicable

30 (viii) POSITION IN GENOME: Not applicable

(ix) FEATURE:

(D) OTHER INFORMATION: 28% identical with
the P14 allergen of birch (Betula verrucosa)

(x) PUBLICATION INFORMATION: ref.14

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

-34-

Ala Gly Trp Asn Ala Tyr Ile Asp Asn Leu Met Ala Asp Gly Thr Cys
1 5 10 15

Gln Asp Ala Ala Ile Val Gly Tyr Lys Asp Ser Pro Ser Val Trp Ala
20 25 30

Ala Val Pro Gly Lys Thr Phe Val Asn Ile Thr Pro Ala Glu Val Gly
35 40 45

5 Ile Leu Val Gly Lys Asp Arg Ser Ser Phe Phe Val Asn Gly Leu Thr
50 55 60

Leu Gly Gly Gln Lys Cys Ser Val Ile Arg Asp Ser Leu Leu Gln Asp
65 70 75 80

Gly Glu Phe Thr Met Asp Leu Arg Thr Lys Ser Thr Gly Gly Ala Pro
85 90 95

10 Thr Phe Asn Ile Thr Val Thr Met Thr Ala Lys Thr Leu Val Leu Leu
100 105 110

Met Gly Lys Gln Gly Val His Gly Gly Met Ile Asn Lys Lys Cys Tyr
115 120 125

Glu Met Ala Ser His Leu Arg Arg Ser Gln Tyr
130 135

15

(7) INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 140 amino acids

20 (B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Not applicable

25 (v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Human (Homo sapiens)

(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

30 (ix) FEATURE:

(D) OTHER INFORMATION: 30% identical with
the P14 allergen of birch (Betula verrucosa)

(x) PUBLICATION INFORMATION: ref. 13

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Gly Trp Asn Ala Tyr Ile Asp Asn Leu Met Ala Asp Gly Thr
1 5 10 15
Cys Gln Asp Ala Ala Ile Val Gly Tyr Lys Asp Ser Pro Ser Val Trp
20 25 30
Ala Ala Val Pro Gly Lys Thr Phe Val Asn Ile Thr Pro Ala Glu Val
35 40 45
5 Gly Val Leu Val Gly Lys Asp Arg Ser Ser Phe Tyr Val Asn Gly Leu
50 55 60
Thr Leu Gly Gly Gln Lys Cys Ser Val Ile Arg Asp Ser Leu Leu Gln
65 70 75 80
Asp Gly Glu Phe Ser Met Asp Leu Arg Thr Lys Ser Thr Gly Gly Ala
85 90 95
10 Pro Thr Phe Asn Val Thr Val Thr Lys Thr Asp Lys Thr Leu Val Leu
100 105 110
Leu Met Gly Lys Glu Gly Val His Gly Gly Leu Ile Asn Lys Lys Cys
115 120 125
Tyr Glu Met Ala Ser His Leu Arg Arg Ser Gln Tyr
130 135 140

15

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 126 amino acids

(B) TYPE: amino acid

20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Not applicable

25 (v) FRAGMENT TYPE: Not applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Yeast

(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

30 (ix) FEATURE:

(D) OTHER INFORMATION: 26% identical with
the P14 allergen of birch (*Betula verrucosa*)

(x) PUBLICATION INFORMATION: ref. 11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

-36-

Met	Ser	Trp	Gln	Ala	Tyr	Thr	Asp	Asn	Leu	Ile	Gly	Thr	Gly	Lys	Val	
1														15		
Asp	Lys	Ala	Val	Ile	Tyr	Ser	Arg	Ala	Gly	Asp	Ala	Val	Trp	Ala	Thr	
				20					25					30		
Ser	Gly	Gly	Leu	Ser	Leu	Gln	Pro	Asn	Glu	Ile	Gly	Glu	Ile	Val	Gln	
				35					40					45		
5	Gly	Phe	Asp	Asn	Pro	Ala	Gly	Leu	Gln	Ser	Asn	Gly	Leu	His	Ile	Gln
								50					55		60	
Gly	Gln	Lys	Phe	Met	Leu	Leu	Arg	Ala	Asp	Asp	Arg	Ser	Ile	Tyr	Gly	
				65		70					75				80	
Arg	His	Asp	Ala	Glu	Gly	Val	Val	Cys	Val	Arg	Thr	Lys	Gln	Thr	Val	
									85		90			95		
10	Ile	Ile	Ala	His	Tyr	Pro	Pro	Thr	Val	Gln	Ala	Gly	Glu	Ala	Thr	Lys
								100		105				110		
Ile	Val	Glu	Gln	Leu	Ala	Asp	Tyr	Leu	Ile	Gly	Val	Gln	Tyr			
				115				120						125		

15 (9) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 125 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Not applicable

(v) FRAGMENT TYPE: Not Applicable

(vi) ORIGINAL SOURCE:

25 (A) ORGANISM: Acanthamoeba

(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

(ix) FEATURE:

(D) OTHER INFORMATION: 25% identical with
30 the P14 allergen of birch (Betula verrucosa)

(x) PUBLICATION INFORMATION: ref. 10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Thr	Trp	Gln	Ser	Tyr	Val	Asp	Thr	Asn	Leu	Val	Gly	Thr	Gly	Ala	Val
1														15	

35	Thr	Gln	Ala	Ala	Ile	Leu	Gly	Leu	Asp	Gly	Asn	Thr	Trp	Ala	Ser	Phe
					20				25				30			

Ala	Gly	Phe	Ala	Val	Thr	Pro	Ala	Gln	Gly	Thr	Thr	Leu	Ala	Gly	Ala
								35				40		45	

Phe Asn Asn Ala Asp Ala Ile Arg Ala Gly Gly Phe Asp L u Ala Gly
50 55 60

Val His Tyr Val Thr Leu Arg Ala Asp Asp Arg Ser Ile Tyr Gly Lys
65 70 75 80

Lys Gly Ala Ser Gly Val Ile Thr Val Lys Thr Ser Lys Ser Ile Leu
85 90 95

5 Val Gly Val Tyr Asn Glu Lys Ile Gln Pro Gly Thr Ala Ala Asn Val
100 105 110

Val Glu Lys Leu Ala Asp Tyr Leu Ile Gly Gln Gly Phe
115 120 125

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(10) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 641 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 5 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA of mRNA

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(v) FRAGMENT TYPE: Not applicable

10 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Phleum pratense

(vii) IMMEDIATE SOURCE:

(A) Pollen from Allergon AB, Engelholm, Sweden

(viii) POSITION IN GENOME: Not applicable

15 (ix) FEATURE: Not applicable

(x) PUBLICATION INFORMATION: Not applicable

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

20	GAAAGCAAAC TTGCAGGACCGAAGATGTCGTGGCAGACGTACGTGGACGAGCACCTGATG	60
	TGCGAGATCGAGGGCCACCACCTCGCTCGCGGCCATCCTCGGCCACGACGGCACCGTC	120
	TGGGCCAGAGCGCCGACTTCCCCCAGTTCAAGCCTGAGGAGATACCGGCATCATGAAG	180
	GATTCGACGGAGCCGGGCACCTCGCCCCCACCGGCATGTTCGTCGCAGGTGCCAAGTAC	240
	ATGGTCATCCAGGGTGAACCGGGTCGCGTCATCCGTGGAAGAAGGGAGCAGGAGGCATC	300
	ACCATAAAGAACGGGCAGGCCTGGTCGTCGGCATCTATGACGAGCCCATGACCCCT	360
25	GGGCAGTGCAACATGGTGGTGGAGAGGGCTTGGCAGTACCTCGTTGAACAAGGCATGTA	420
	ACTGGCTGATCCATGGCTCCACGTCTCCACGATCGATGATGATCATACAGTTTCACG	480
	TTCTTTAACATCTATTGGAATATATGGGGCTCTCCTCTTACCGGCTCTGGTCA	540
	TGGATCACTGATGACCAAGTTGCTCTGGAAGTTCAATTGTAATGCCATCTGGCTTCTA	600
	TCTTCTTCAATGTTTTTTCTTTCGGTTAAAAAAA	641

(11) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 393 nucleotides

(B) TYPE: nucleic acid

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA of mRNA, coding region

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

10 (v) FRAGMENT TYPE: Not applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Phleum pratense

(vii) IMMEDIATE SOURCE:

(A) Pollen from Allergon AB, Engelholm, Sweden

15 (viii) POSITION IN GENOME: Not applicable

(ix) FEATURE: Not applicable

(x) PUBLICATION INFORMATION: Not applicable

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

20	ATGTCGTGGCAGACGTACGTGGACGAGCACCTGATGTGCGAGATCGAGGGCCACCACCTC	60
	GCCTCGGCCGCCATCCTCGGCCACGACGGCACCGTCTGGGCCAGAGCGCCGACTTCCCC	120
	CAGTTCAAGCCTGAGGAGATCACCGGCATCATGAAGGATTTCGACGAGCCGGGCACCTC	180
	GCCCCCACCGGCATGTTCGTCGCAAGGTGCCAGTACATGGTCATCCAGGGTGAAACCCGGT	240
25	CG-JTCATCCGTGGCAAGAAGGGAGCAGGAGGCATCACCATAAAAGAACGACCGGGCAGGCG	300
	CTGGTCGTGGCAGTACGAGCCCAGACCCCTGGCAGTGCAACATGGTGGTGGAG	360
	AGGCTTGGCAGTACCTCGTTGAACAAGGCATG	393

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(12) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 131 amino acid residues

(B) TYPE: amino acid

5 (C) STRANDEDNESS: Not applicable

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide, P14(T) allergen

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Not applicable

10 (v) FRAGMENT TYPE: Not applicable

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Phleum pratense

(vii) IMMEDIATE SOURCE: Not applicable

(viii) POSITION IN GENOME: Not applicable

15 (ix) FEATURE: Amino acid identity with P14 allergen from Betula
verrucosa is 77%

(x) PUBLICATION INFORMATION: Not applicable

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

1	5	10	15	
20	Met Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Cys Glu Ile	20	25	30
	Glu Gly His His Leu Ala Ser Ala Ala Ile Leu Gly His Asp Gly	35	40	45
25	Thr Val Trp Ala Gln Ser Ala Asp Phe Pro Gln Phe Lys Pro Glu	50	55	60
	Glu Ile Thr Gly Ile Met Lys Asp Phe Asp Glu Pro Gly His Leu	65	70	75
	Ala Pro Thr Gly Met Phe Val Ala Gly Ala Lys Tyr Met Val Ile	80	85	90
30	Gln Gly Glu Pro Gly Arg Val Ile Arg Gly Lys Lys Gly Ala Gly	95	100	105
	Gly Ile Thr Ile Lys Lys Thr Gly Gln Ala Leu Val Val Gly Ile	110	115	120
35	Tyr Asp Glu Pro Met Thr Pro Gly Gln Cys Asn Met Val Val Glu	125	130	
	Arg Leu Gly Asp Tyr Leu Val Glu Gln Gly Met			

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What is claimed is:

1. A recombinant DNA molecule, comprising DNA coding for a polypeptide having at least one epitope of a P14 allergen of grass, weeds and trees of the order Fagales.

5

2. A recombinant DNA molecule according to claim 1, wherein the allergen is selected from P14 allergens of the group consisting of birch, alder, hazel, hornbeam and oak.

10

3. A recombinant DNA molecule according to claim 1, wherein the allergen is a P14 allergen of birch.

15

4. A recombinant DNA molecule according to claim 1, 2 or 3, which codes for a polypeptide having the entire amino acid sequence of the P14 allergen.

20

5. A recombinant DNA molecule according to claim 3, which codes for a polypeptide having all or part of the amino acid sequence as defined in the Sequence Listing by SEQ ID NO:3.

25

6. A replicable microbial expression vehicle capable, in a transformant host organism, of being replicated and of directing expression of a DNA of claims 1, 2, 3 or 5 to produce said polypeptide.

30

7. A replicable microbial expression vehicle capable, in a transformant host organism, of being replicated and of directing expression of a DNA of claim 4 to produce said polypeptide.

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8. A prokaryotic or eukaryotic host organism transformed with a microbial expression vehicle capable, in said organism, of being replicated and of directing

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expression of a DNA of claim 1, 2, 3 or 5 to produce said polypeptide.

9. A prokaryotic or eukaryotic host organism transformed with a microbial expression vehicle capable, 5 in said organism, of being replicated and of directing expression of a DNA of claim 4 to produce said polypeptide.

10. A host organism according to claim 8, 10 wherein the host organism is Escherichia coli.

11. A host organism according to claim 9, wherein the host organism is Escherichia coli.

15 12. A method for producing a polypeptide having at least one epitope of a P14 allergen, comprising culturing a host organism containing a microbial expression vehicle capable, in said host organism, of being replicated and of directing expression of a DNA of 20 claim 1, 2, 3 or 5 to produce said polypeptide.

13. A method for producing a polypeptide having at least one epitope of a P14 allergen, comprising culturing a host organism containing a microbial 25 expression vehicle capable, in said host organism, of being replicated and of directing expression of a DNA of claim 4 to produce said polypeptide.

14. A composition comprising a polypeptide 30 having at least one epitope of a P14 allergen and produced by a method according to claim 12.

15. A composition comprising a polypeptide 35 having at least one epitope of a P14 allergen and produced by a method according to claim 13.

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16. A composition comprising a polypeptide having at least one epitope of a P14 allergen and produced by chemical synthesis according to the whole or a part of SEQ ID NO:3.

5 17. A method for detecting antibodies, comprising contacting serum of a mammal with a composition according to claim 14, and detecting any immunological reaction between antibodies in the serum and said polypeptide having at least one epitope of a P14 allergen.

10 18. A method according to claim 17, wherein the detected antibody is an IgE antibody.

15 19. A method for detecting antibodies, comprising contacting serum of a mammal with a composition according to claim 15, and detecting any immunological reaction between antibodies in the serum and said polypeptide having at least one epitope of a P14 allergen.

20 20. A method according to claim 19, wherein the detected antibody is an IgE antibody.

25 21. A method for detecting antibodies, comprising contacting serum of a mammal with a composition according to claim 16, and detecting any immunological reaction between antibodies in the serum and said polypeptide having at least one epitope of a P14-allergen.

30 22. A method according to claim 21, wherein the detected antibody is an IgE antibody.

35 23. A method for detecting in vitro the cellular reaction to P14 allergen, comprising contacting mammalian cells with a composition according to claim 14, and detecting the reaction f said cells.

24. A method for detecting in vitro the cellular reaction to P14 allergen, comprising contacting mammalian cells with a composition according to claim 15, and detecting the reaction of said cells.

5 25. A method for detecting in vitro the cellular reaction to P14 allergen, comprising contacting mammalian cells with a composition according to claim 16, and detecting the reaction of said cells.

10 26. A method for treating a mammal afflicted with a pollen allergy, comprising administering an effective amount of a composition according to claim 14 to hyposensitize said mammal.

15 27. A method for treating a mammal afflicted with a pollen allergy, comprising administering an effective amount of a composition according to claim 15 to hyposensitize said mammal.

20 28. A method for treating a mammal afflicted with a pollen allergy, comprising administering an effective amount of a composition according to claim 16 to hyposensitize said mammal.

25 29. A method for detecting allergic reactions to a P14 allergen, comprising administering a composition according to claim 14, so as to elicit bronchial, conjunctival, dermal, nasal or oral provocation of a mammal, and detecting any immunological reaction between 30 said organs and said polypeptide.

30 35 30. A method for detecting allergic reactions to a P14 allergen, comprising administering a composition according to claim 15, so as to elicit bronchial, conjunctival, dermal, nasal or oral provocation of a

mammal, and detecting any immunological reaction between said organs and said polypeptide.

31. A method for detecting allergic reactions to a P14 allergen, comprising administering a composition
5 according to claim 16, so as to elicit bronchial, conjunctival, dermal, nasal or oral provocation of a mammal, and detecting any immunological reaction between said organs and said polypeptide.

10 32. A method for detecting the presence of a P14 allergen, comprising, contacting a sample suspected of containing a P14 allergen with poly(L-proline), and detecting any reaction between any P14 allergen and the poly(L-proline).

15 33. A method for purifying a P14 allergen or a polypeptide having the antigenicity of a P14 allergen, comprising binding any P14 allergen in a sample to poly(L-proline), and collecting the bound P14 allergen.

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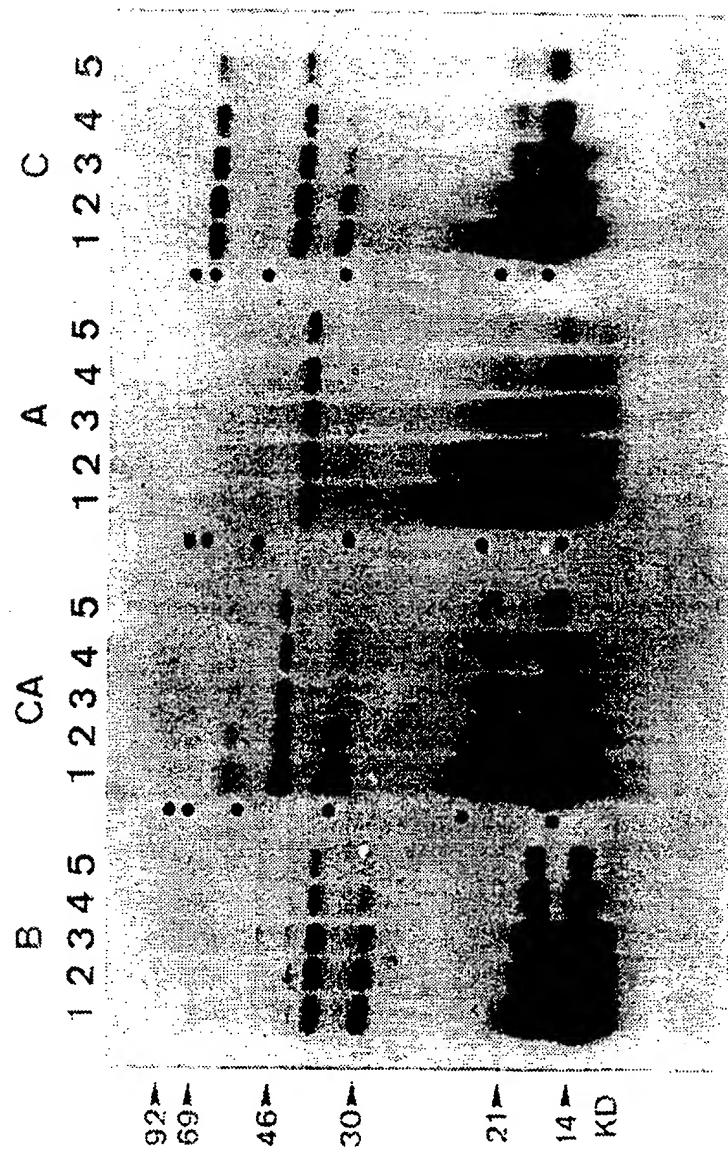


FIG.1A

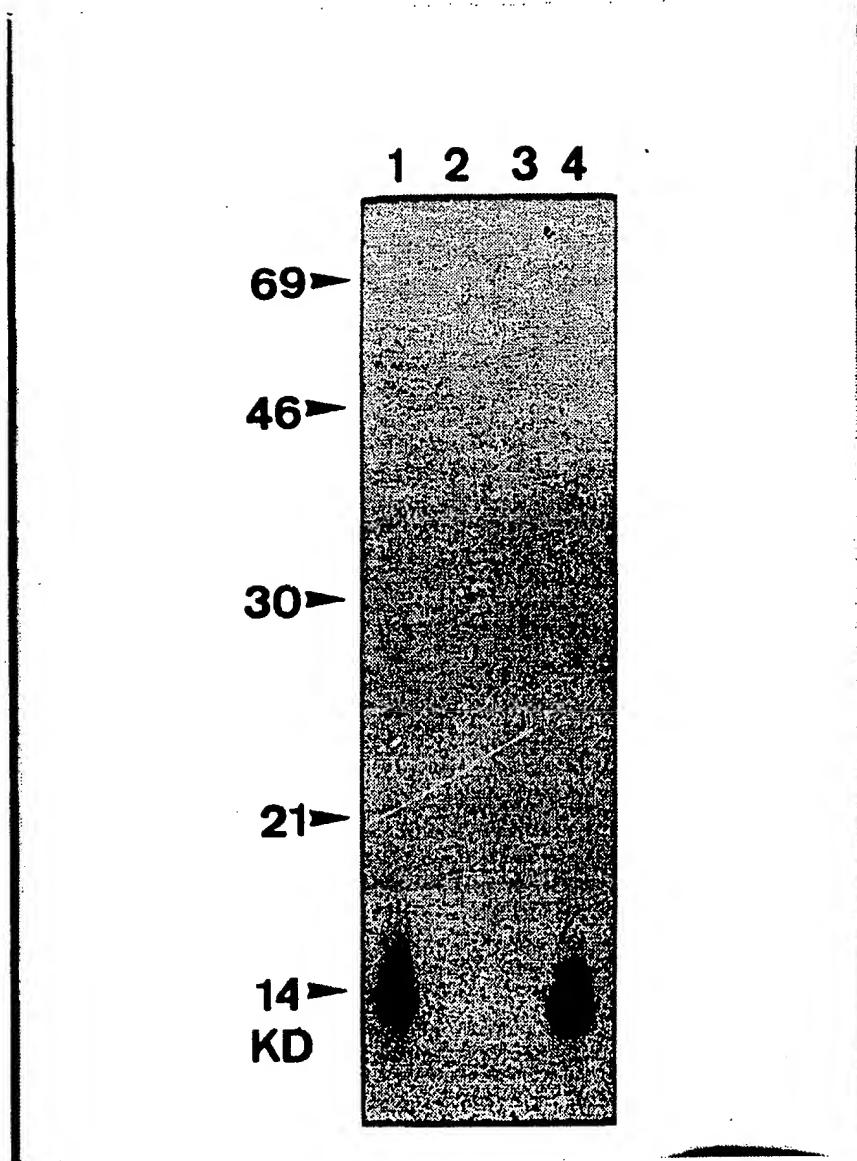


FIG.1B

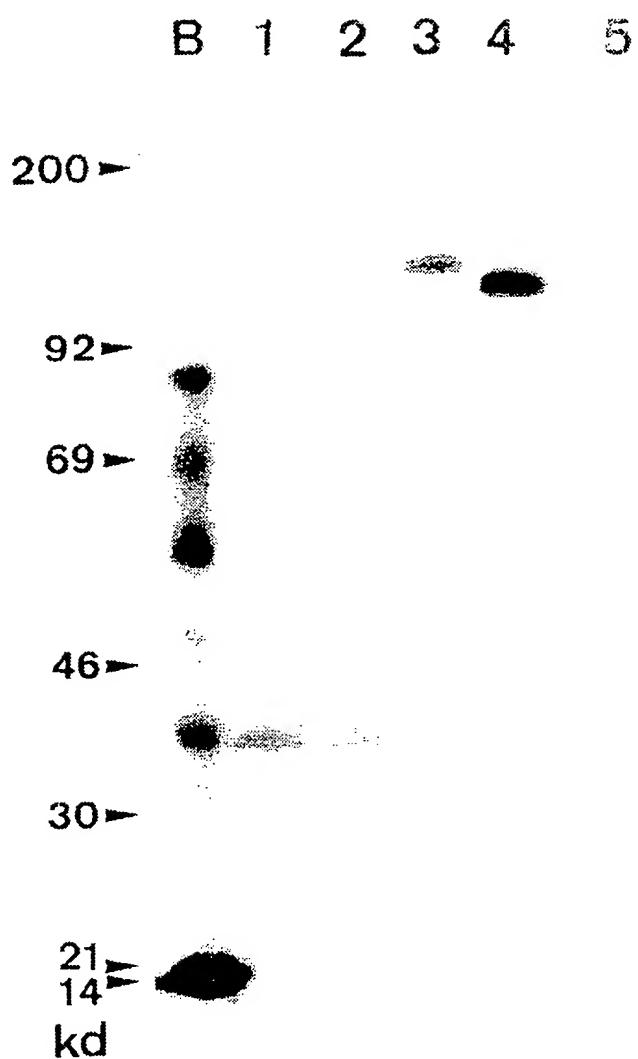


FIG.2

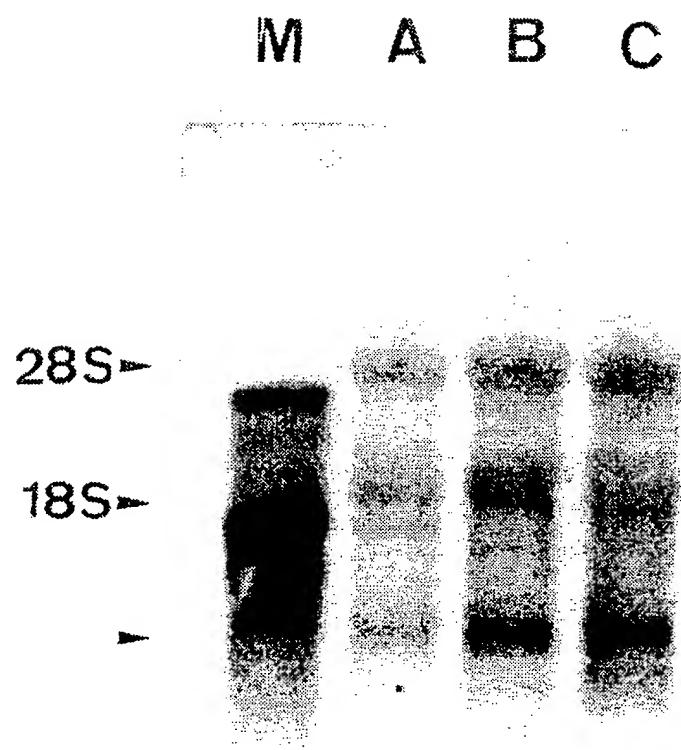


FIG.3

SUBSTITUTE SHEET

10	20	30	40	50	60
CAGAGAAAGCGAAAGCTCTCGGCCACAACAAAACGAAGTAGAAGAAGAGTGAGCAAG					
70	79				
AGACAGAGGGAGAGGAAA					
90	100	110	120		
ATG TCG TGG CAA ACG TAC GTG GAT GAA CAT TTG ATG TGC GAT ATC					
met ser trp gln thr tyr val asp glu his leu met cys asp ile					
130	140	150	160		
GAC GGG CAA GCC AGC AAC TCG CTG GCA TCT GCG ATC GTC GGT CAC					
asp gly gln ala ser asn ser leu ala ser ala ile val gly his					
180	190	200	210		
GAT GGC TCT GTG TGG GGC CAG AGC TCT TCC TTC CCA CAG TTT AAG					
asp gly ser val trp ala gln ser ser ser phe pro gln phe lys					
220	230	240	250		
CCT CAG GAA ATC ACT GGT ATC ATG AAG GAC TTT GAG GAG COG GGT					
pro gln glu ile thr gly ile met lys asp phe glu glu pro gly					
270	280	290	300		
CAT CTT GCT CCG ACG GGC TTA CAC CTT GGG GGC ATA AAA TAC ATG					
his leu ala pro thr gly leu his leu gly gly ile lys tyr met					
310	320	330	340		
GTC ATC CAG GGA GAG CCT GGT GCT GTC ATC CGT GGA AAG AAG GGA					
val ile gln gly glu ala gly ala val ile arg gly lys lys gly					
360	370	380	390		
TCT GGA GGT ATT ACT ATA AAG AAG ACT GGT CAA GCT CTC GIT TTT					
ser gly gly ile thr ile lys lys thr gly gln ala leu val phe					
400	410	420	430		
GGC ATC TAT GAA GAG CCT GTG ACA <u>CCA GGA CAG TGC AAC ATG GIT</u>					
gly ile tyr glu glu pro val thr pro gly gln cys asn met val					
450	460	470	480		
<u>GTT GAG AGG TTG GGG GAT TAC CTT ATT GAC CAG GGC CTG TAG</u>					
val glu arg leu gly asp tyr leu ile asp gln gly leu *					
490	500	510	520	530	540
GCAAAGGTCTATCATCATTTGGGGCTTAATTGTTTTTTTTTTGCCTTATTTOCC					
550	560	580	590	600	610
TTTGTATTGGTCCAAGTGTGCATOGATCTCATTTGAAAGCCTAAATTGGCAGTGAA					
620	630	640	650	660	670
GTTGTTGCAGACAATAACCATGIGAGAACTAAAACATTGICCTGIGTTGGTTGTTGAA					
680	690	700	710		
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA					

FIG. 4

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	10	20	30	40	50	60	70	80
Mouse	MAGWNAYIDS. IMAD. .G...TCQDAATIVGYKDSPSWAAVPGRETFSITPAEVGVLVG..KDRSSFFVNGLTLLGGQKCS							
Bovine	AGWNAYIDN. IMAD. .G...TCQDAATIVGYKDSPSWAAVPGCIFVNNTPAEVGVLVG..KDRSSFFVNGLTLLGGQKCS							
Human	MAGWNAYIDN. IMAD. .G...TCQDAATIVGYKDSPSWAAVPGCIFVNNTPAEVGVLVG..KDRSSFFVNGLTLLGGQKCS							
White Birch	MS.WQRYYDEHLMCDIGQASNSLASSAIVHGHDGS..WMA..QSSESEPKPOELTGIMDDEPCHIAPTPGLHUGGTRIM							
Yeast	MS.WQAYTDN.LIGT..G...KVDKAVIYSRAG.DAWMATSGG..LSIQPNETIGEIVQGEDNPAGLQSNGLHQGQKF.							
Acanthamoeba	..TWSQSYDVTNLVGT..G...AVTQAALIGELDENT..WASFAG..FAVTPAQGTTLAGATNTDAIRAGEFDLAGVHIV							
	90	100	110	120	130	140		
Mouse	VIRDSSLQDGEEFTMDIRTKSTGGAPTENVTVTAKTLVILMKEGVHGGCLINKKCYEMASHLRSQY							
Bovine	VIRDSSLQDGEEFTMDIRTKSTGGAPTENVTVTAKTLVILMKEGVHGGCLINKKCYEMASHLRSQY							
Human	VIRDSSLQDGESMDIRTKSTGGAPTENVTVTAKTLVILMKEGVHGGCLINKKCYEMASHLRSQY							
White Birch	VI....Q.GEAGAYTRKGKGSGG.....ITIKKTCQALVEGIKEEPVTPQOCMVVERLGQYLD.QGL							
Yeast	...MLRADDRTSYGRHDAG...WCVVRTKQTVTIAHTPPPTVQAGEATKLVQLADYLIGVQX							
Acanthamoeba	T....LRADDRTSYGRKGASG.....VITVVRTKTSILVGVINERKTOPGTAANVERTADYLIG.QGF							

FIG. 5

1 2 3 4 5 6 7 8 9

200-
92-
69-
46-
30-
21-
14-
KD

FIG.6

SUBSTITUTE SHEET

A B C D E F G H I J K L M N O P Q R

200-

92-

69-

46-

30-

21-

14-

KD

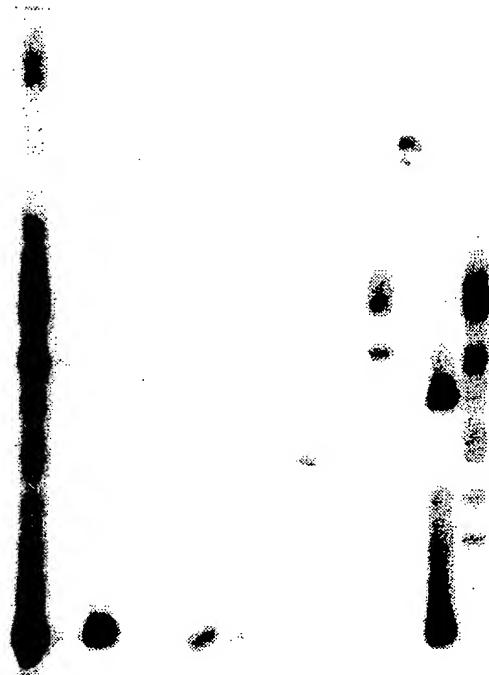


FIG.7

SUBSTITUTE SHEET

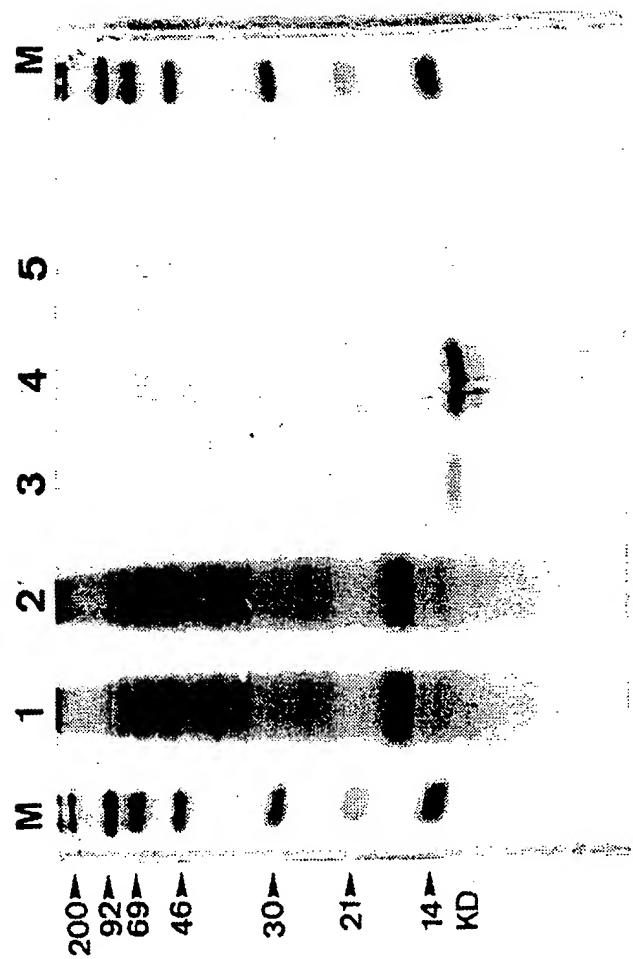


FIG.8

SUBSTITUTE SHEET

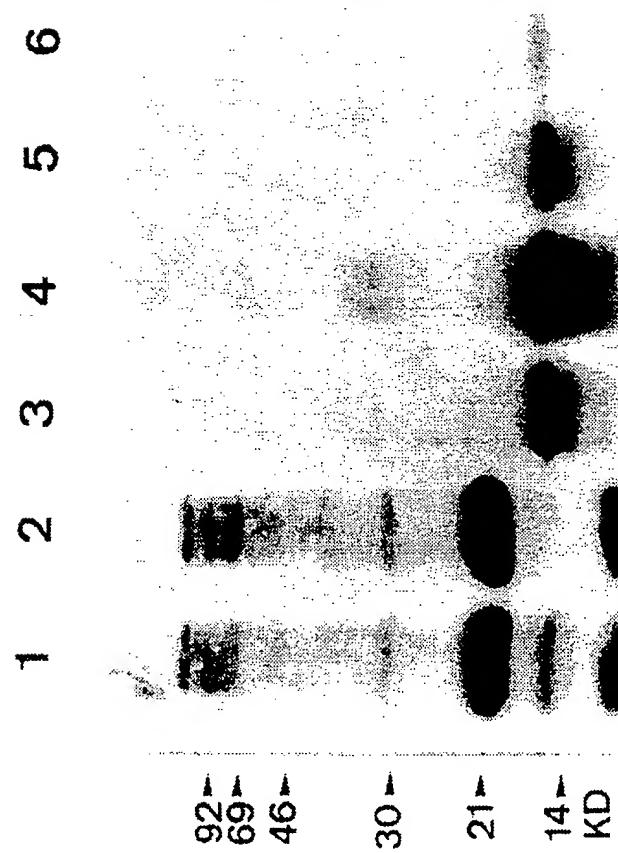


FIG.9

SUBSTITUTE SHEET

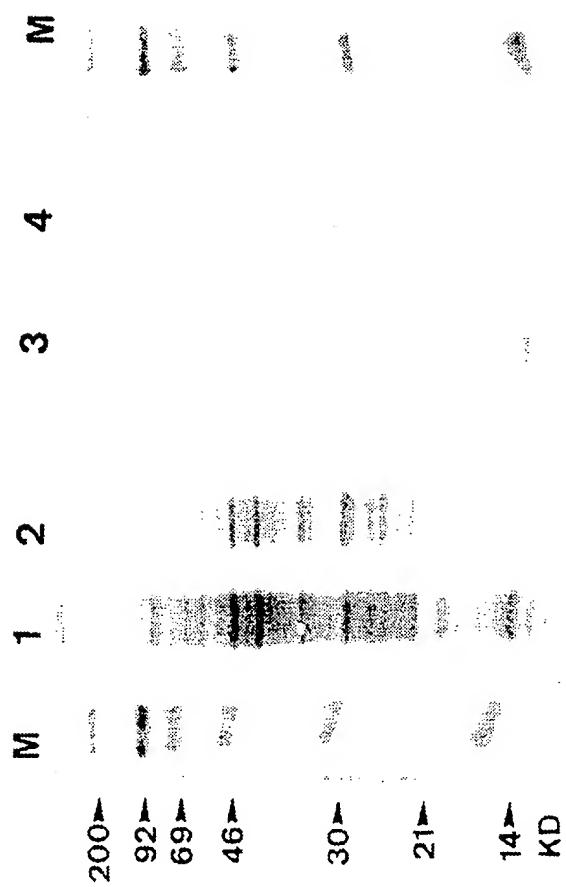


FIG.10

SUBSTITUTE SHEET

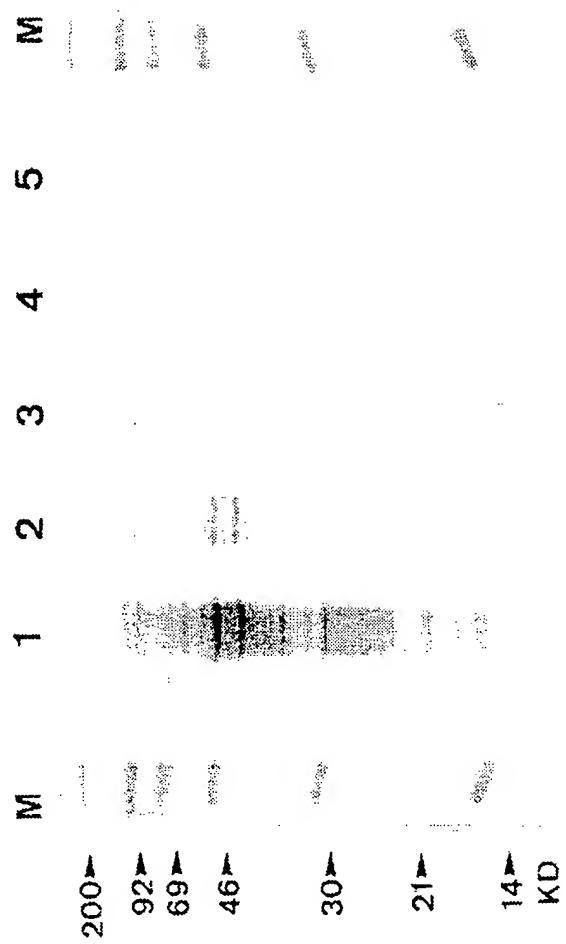


FIG.11

SUBSTITUTE SHEET

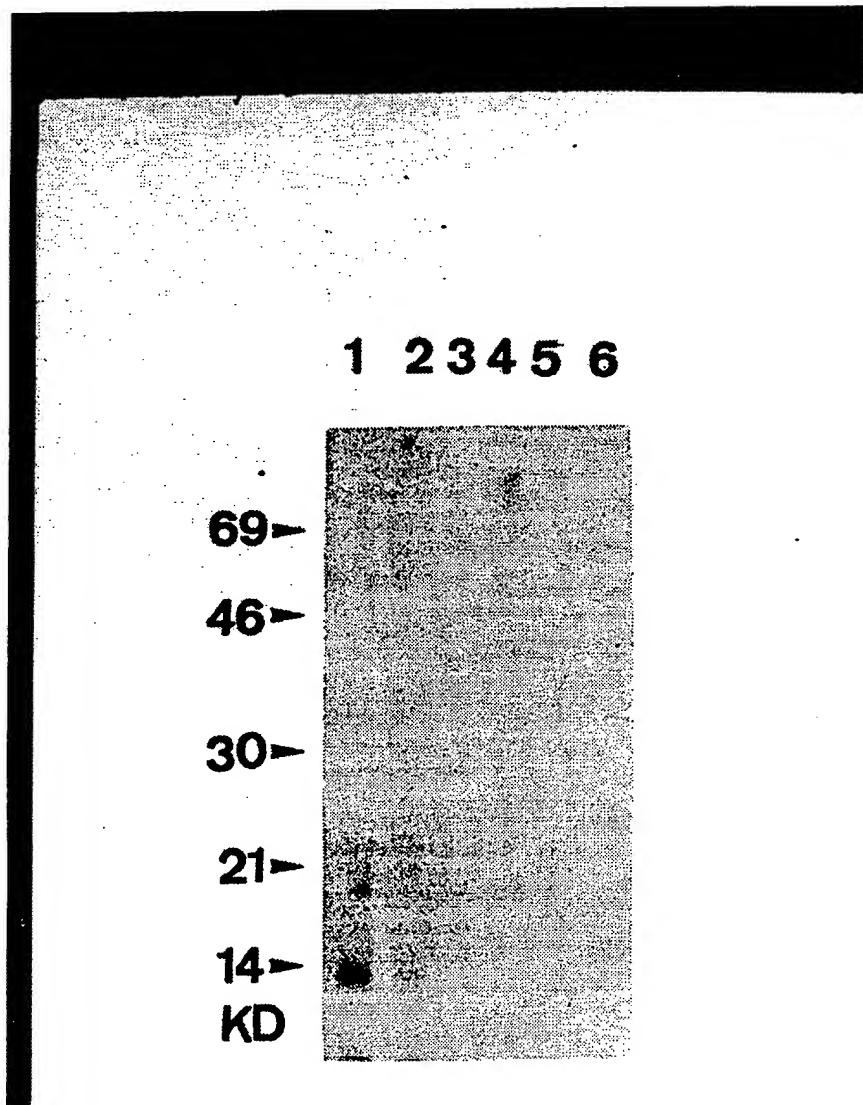


FIG.12

SUBSTITUTE SHEET

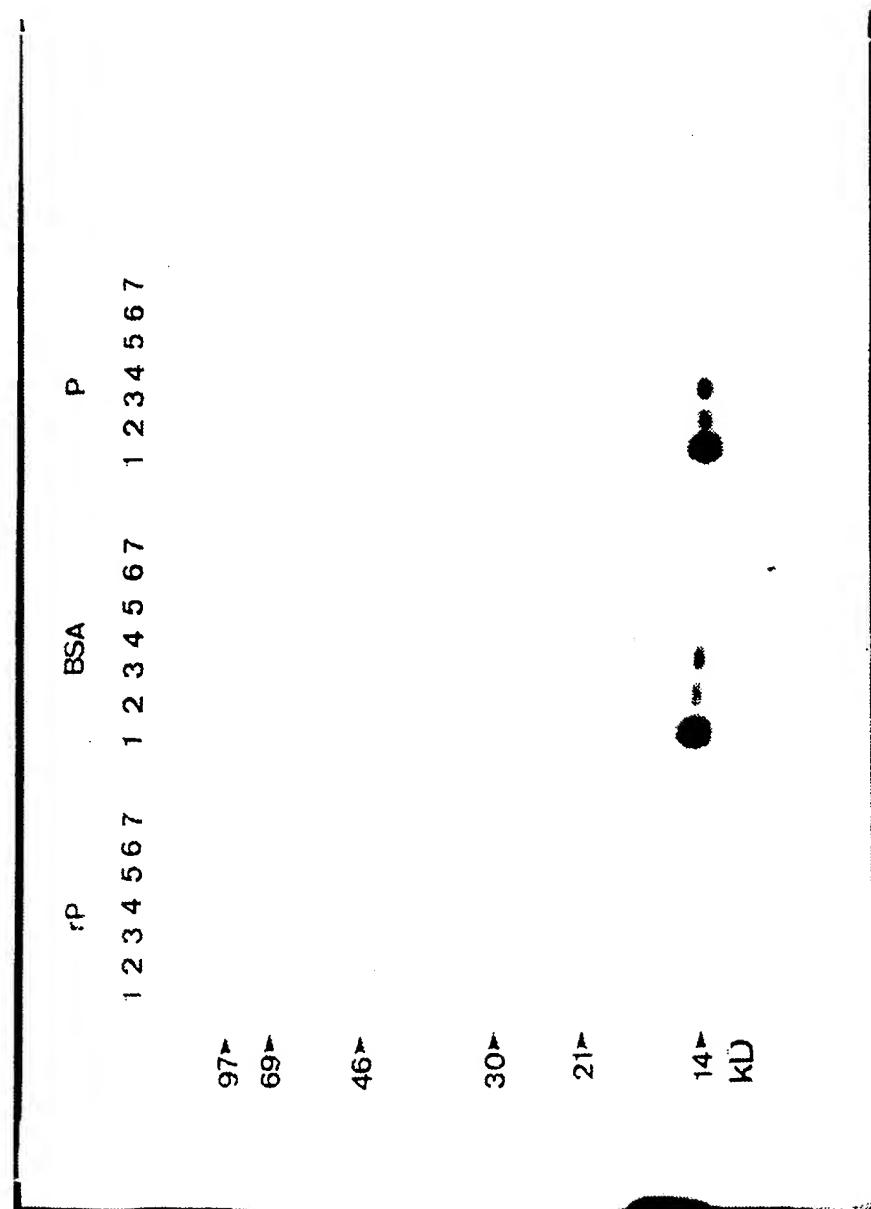


FIG.13

SUBSTITUTE SHEET

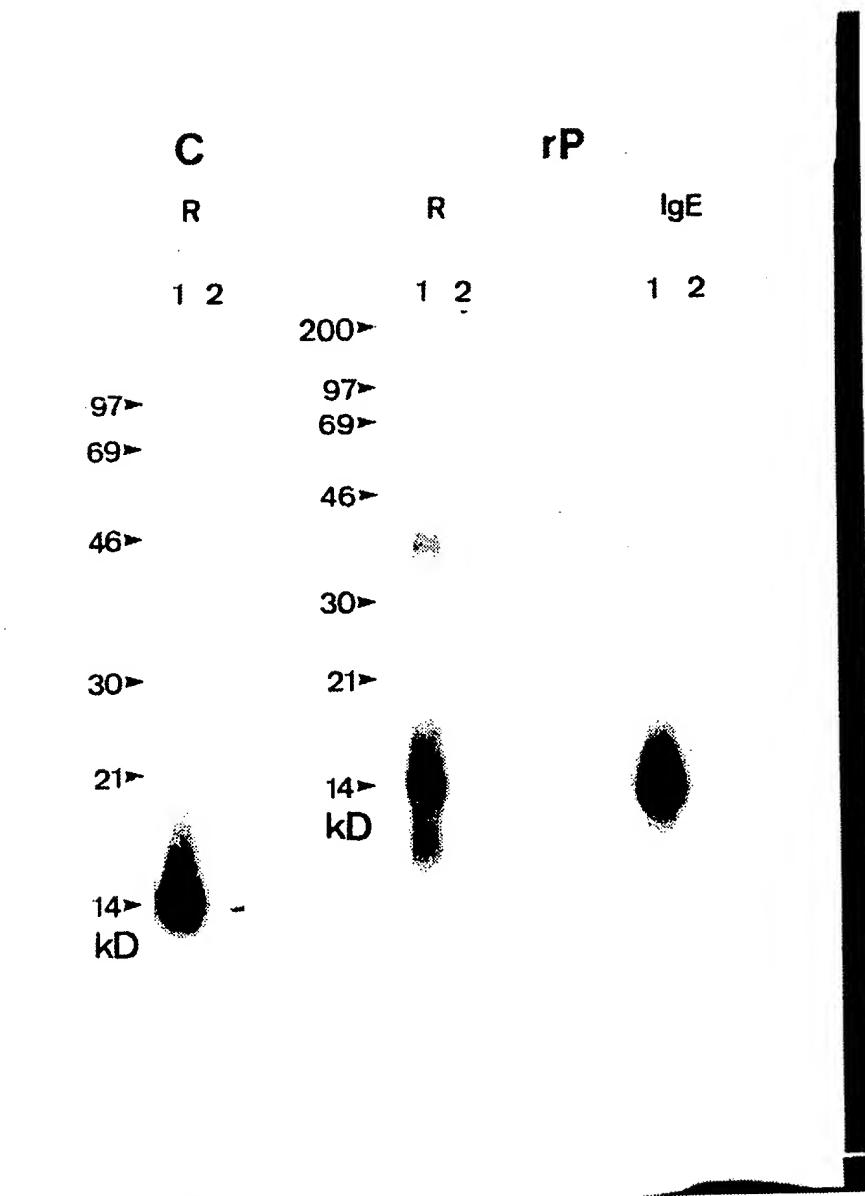


FIG.14

SUBSTITUTE SHEET

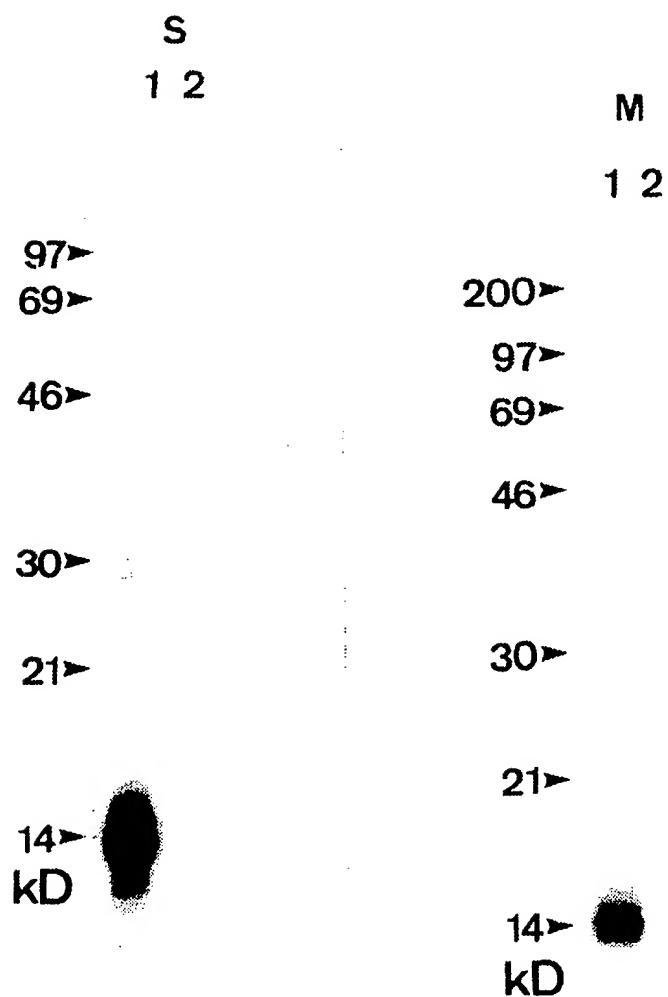


FIG.15

17/18

10 20

GAAAGCAAACITGCAGGACCGAAG

30

40

50

60

ATG TCG TGG CAG ACG TAC GIG GAC GAG CAC CTG ATG TGC GAG AIC
met ser trp gln thr tyr val asp glu his leu met cys glu ile

80

90

100

110

GAG GGC CAC CAC CTC GCC TCG GCG GGC ATC CTC GGC CAC GAC GGC
glu gly his his leu ala ser ala ala ile leu gly his asp gly

120

130

140

150

ACC GTC TGG GCC CAG AGC GCC GAC TTC CCC CAG TTC AAG CCT GAG
thr val trp ala gln ser ala asp phe pro gln phe lys pro glu

170

180

190

200

GAG ATC ACC GGC ATC ATG AAG GAT TTC GAC GAG CCG GGG CAC CTC
glu ile thr gly ile met lys asp phe asp glu pro gly his leu

210

220

230

240

GCC CCC ACC GGC ATG TTC GTC GCA GGT GCC AAG TAC ATG GTC ATC
ala pro thr gly met phe val ala gly ala lys tyr met val ile

260

270

280

290

CAG GGT GAA CCC GGT CGC GTC ATC CGT GGC AAG AAG GGA GCA GGA
gln gly glu pro gly arg val ile arg gly lys lys gly ala gly

300

310

320

330

GGC ATC ACC ATA AAG AAG ACC GGG CAG GCG CTG GTC GTC GCC ATC
gly ile thr ile lys lys thr gly gln ala leu val val gly ile

350

360

370

380

TAT GAC GAG CCC ATG ACC CCT GGG CAG TGC AAC ATG GTG GTG GAG
tyr asp glu pro met thr pro gly gln cys asn met val val glu

390

400

410

420

AGG CTT GGC GAC TAC CTC GTT GAA CAA GGC ATG TAG
arg leu gly asp tyr leu val glu gln gly met *

430

440

450

460

470

480

ACTGGCTGATCCATGGCTTOCACGCTCOCAOGATCGATGATGATCATACAGTTTCAOG

490

500

510

520

530

540

TTCCTTTAACATCTATTGGAATATATATGGGGCTCTCCTCTTACCGGCTCTGGCTCA

550

560

570

580

590

600

TGGATCACTGATGACCAGTTGCTCTGGAAGTTCAATTGTAATGCCATCTGGCTTCTA

610

620

630

640

TCTTCTCAATGTTTTTCITTCGGTTAAAAAAAAAA

FIG. 16



FIG.17

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 91/01513

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC
 Int.C1.5 C 12 N 15/29 C 07 K 13/00 C 12 N 15/70
 G 01 N 33/53 C 12 Q 1/68 A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched⁷

Classification System	Classification Symbols		
Int.C1.5	C 12 N C 12 Q	C 07 K A 61 K	G 01 N

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
O,X	Clinical Experimental Allergy, volume 20, 1990, suppl. 1, meeting 8-11 July 1990, S. d'Abusco et al.: "Characterization of cDNA for Parietaria pollen allergens", page 48, see abstract OP52 (cited in the application) ---	1,6-8, 10,12, 14,17, 18-20
X	Biochimica et Biophysica Acta, volume 967, 1988, Elsevier, U. Lindberg et al.: "The use of poly(L-proline)-sepharose in the isolation of profilin and profilactin complexes", pages 391-400, see the whole document ---	32,33 -/-

¹⁰ Special categories of cited documents :¹⁰

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "R" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

13-11-1991

Date of Mailing of this International Search Report

06.01.92

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

Nicole De Bie

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	Int. Arch. Allergy Appl. Immunol., volume 98, 1988, H. Breiteneder et al.: "Isolation and characterization of messenger RNA from male inflorescences and pollen of the white birch (<i>Betula verrucosa</i>), pages 19-24, see page 21, left-hand column, "Immunoblotting of aqueous BV pollen extracts" ---	1-31
Y	The EMBO Journal, volume 8, no. 7, July 1989, (Eynsham, Oxford, GB) H. Breiteneder et al.: "The gene coding for the major birch pollen allergen Betv1, is highly homologous to a pea disease resistance response gene", pages 1935-1938, see the whole article ---	1-31
P,X	Science, volume 253, 2 August 1991, R. Valenta et al.: "Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals", pages 557-560, see the whole article ---	1-32
A	Allergy, volume 44, no. 6, August 1989, E. Jarolim et al.: "IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of <i>Betula verrucosa</i> ", pges 385-395, see the whole article ---	1-31
A	Allergy, volume 45, no. 6, August 1990, T. Birkner et al.: "Evaluation of immunotherapy-induced changes in specific IgE, IgG and IgG subclasses in birch pollen allergic patients by means of immunoblotting", pages 418-426, see the whole article ---	17-22, 26-28
A	DE,A,3147763 (STICKL) 9 June 1983, see the whole document -----	23-25

FURTHER INFORMATION - CONTINUED FROM THE SECOND SHEET

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V. OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 26-28 and 29-31 at least partially are directed to a method of treatment of diagnostic method practised on the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this International application as follows:

1. As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- The additional search fees were accompanied by applicant's protest.
- No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

EP 9101513
SA 50094

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 20/12/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A- 3147763	09-06-83	None	-----